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Antioxidant and Antimicrobial Potentials of *Artemisia Indica* Collected from the Nepal Region

Akhtar Nahid[1], Choudhury Neelabh[1] *Kumar Navneet[2, 3]

[1]*Department of Molecular Biology and Genetics, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab – 144411*
[2]*Department of Biochemistry, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab – 144411*
[3]*Department of Biochemistry, School of Life Sciences, Central University of Rajasthan, Bandar Sindri, Ajmer (District), Kishangarh, Rajasthan - 305801*

Abstract

Artemisia indica is traditionally used in Thai medicine, Chinese medicine and by locals in different parts of India, Nepal, Pakistan and various other regions of South-east Asia. The study aimed to evaluate the antioxidant, antimicrobial and phytochemical constituents of the methanol extract of *A. indica* which may infer the antioxidant and antibacterial activity of these plants. DPPH radical scavenging, ferric reducing power assay, total antioxidant assay, total phenol content assay and total flavonoid content assay were done to determine the antioxidant activity of the plant extract. Agar gel diffusion method was used to determine the antibacterial properties against *Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli, Salmonella enterica, and Pseudomonas putida* in a dose-dependent manner. The plant extract was found to show significant antibacterial and anti-oxidant properties. The presence of phenol and flavonoid compounds present in the plant could be the reason behind the antioxidant and antibacterial activity. The current study shows that *A. indica* could be a good source for preparation of antibacterial and antioxidant preparations in future.

Keywords: *Artemisia indica*, antioxidant, antimicrobial, free radicals, plant extract

INTRODUCTION

Sustaining life in absence of oxygen is impossible. But the importance of oxygen is contradicted by deleterious effects of free radicals and reactive oxygen species like hydroxyl radical, superoxide anion radical, and hydrogen peroxide generated from oxygen. Free radicals are highly reactive molecules having an unpaired electron which are produced inside cells during different metabolic processes such as reactions involved in respiration, inflammation, phagocytosis, and arachidonate pathway [1]. Free radicals are also produced by exposure to cigarette smoke, ozone, environmental pollutants, and radiation. These molecules initiate a cascade of reaction causing cellular damage and disruption of homeostasis by damaging essential biomolecules like DNA, lipid, and proteins [2, 3]. Reactive oxygen species also induce apoptosis, necrosis, oxidation of low-density lipoproteins and oxidative stress [4]. Though ROS are necessary for protection against viruses and bacteria, oxidative stress has fatal consequences [5]. Studies have inferred that these molecules are associated with initiation and progression of cancer, arthritis, heart diseases, atherosclerosis, aging, parkinson's disease, alzheimer's disease and a myriad of other inflammatory diseases [6, 7].

Antioxidants scavenge the reactive oxygen species, thus inhibiting the oxidation of different macromolecules and eventually preventing further cellular damage [8]. As ROS produced under oxidative stress have serious consequences, our body has developed an efficient antioxidant mechanism comprising of antioxidant enzymes like superoxide dismutase, glutathione peroxidase, and catalase. Antioxidants can also be supplemented exogenously to curb oxidative stress [9]. The major sources of these exogenous antioxidants are fruits, vegetables, and herbs. The antioxidant activity of various plants has been evaluated, and moat of the plants have antioxidant properties on the virtue of a plethora of phytochemicals present in them [10-13]. Also, new sources of potent antimicrobial agents need to be identified because of toxic effects of currently used drugs and development of resistance in microbes to antibiotics [14]. The excessive and careless use of antibiotics for the treatment of infectious diseases has led to the development of several antibiotic-resistant fungus and bacteria. Due to the emergence of new antibiotic-resistant microbes, scientists globally are studying the pharmacological properties of plants and herbs to identify and isolate novel antimicrobial compounds. Plants can be a better and cheaper substitute to the commercial synthetic drugs which have different side effects. Also, plants are believed to be safe, less toxic than synthetic drugs and have many pharmacological activities [15]. Traditionally spices and herbs are used as a preservative which suggests that they are rich in antimicrobial and antioxidant compounds. The isolation of these compounds can lead to the new therapies for the treatment of neurodegenerative diseases, inflammatory diseases, and prevention of several bacterial and fungal infections.

In this study, the phytochemical constituents, antioxidant activity and antimicrobial activity of *Artemisia indica* have been evaluated considering its widespread use in traditional medicine globally (Figure 1). *A. indica* is a medicinally important perennial shrub belonging to Aseraceae family which grows wildly in South Asia and other cold temperate region of Asia [14, 16-18]. It is vernacularly called "Titepati" in Darjeeling, India, and Nepal whereas

mugwort and Indian wormwood are other common names for this plant [17, 19]. It has been an integral part of traditional medicine in Nepal, India, and Thailand. In Thailand, it is used for the treatment of malaria whereas in Nepal it is used for the treatment of skin and gastrointestinal diseases [14, 20]. In different regions of India, it is used as a cure for asthma, amoebic dysentery, helminthic infection and as a laxative [18]. Antimalarial compounds such as exiguaflavanone, artemisin, and maacklain have been isolated from this plant [20, 21]. Several other phytochemicals such as davanone, casticin, elemene, farnesene, oleanic acid, ascaridole and carnosol are present in this plant [14, 20, 22]. Essential oil of *A. indica* has shown anticancer activity in human cell lines of breast cancer, hepatocarcinoma, lung cancer and colon cancer [14, 19]. Compounds isolated from *A. indica* have positively modulated the GABA-A receptors in mice inferring that they can be used for the treatment of epilepsy and depression [22]. Antidiabetic, anti-inflammatory and antihelminthic properties of *A. indica* have been reported [23-25].

The antioxidant activity, antimicrobial activity and phytochemical constituents of this plant were measured considering the role of this plant in Ayurveda and ethnomedicine across the globe. Antioxidant activity was determined using DPPH, total antioxidant activity and reducing power assay. The quantitative phytochemical analysis was done to determine total phenolic and total flavonoid content. The antibacterial activity of the plant extract was determined by agar well diffusion method against five bacterial strains, two-gram positive bacteria (*Streptococcus pyogenes, Staphylococcus aureus*) and three Gram-negative bacteria (*Escherichia coli, Salmonella enterica, and Pseudomonas putida).*

Figure 1: Leaves and flowers of *Artemisia indica*

MATERIALS AND METHODS

Sample Collection and Extract Preparation

Leaves of *Artemisia indica* were collected from Simara, Bara, Nepal (Latitude: 27.1602, Longitude: 84.9796) in the first week of January 2017. The leaves were washed to remove dust and soil and dried in hot air oven. The dried leaves were powdered using a mechanical grinder, and the extract was prepared in 90% methanol using Soxhlet apparatus. The extract was dried and kept in the refrigerator for further analysis.

Total Phenol Content

Folin Coilcateau method was used to measure the total phenolic content of *A. indica* [26]. Plant extract (200 µl of 1mg/ml) was mixed with 2 % Na_2CO_3 (2ml) and 10 % Folin Coicateau reagent (2.5 ml). The reaction mixture was then incubated for 15 minutes at 45^0C in a water bath. Absorbance was taken at 765 nm. Gallic acid was used as a standard phytochemical.

Total Flavonoid Content

The flavonoid content of *A. indica* was determined by following aluminum chloride reducing method [27]. Plant extract of *A. indica* (0.2 ml of 300µg/ml) was mixed with 0.6 ml methanol. After that, aluminum chloride (40 µl, 10 %), Potassium acetate (40 µl, 1 M) and distilled water (1120 µl) were added in the mentioned order and incubated for 30 minutes at room temperature. Absorbance was taken at 420 nm using ELICO SL 210 UV-VIS spectrophotometer. Quercetin was used as a standard phytochemical.

DPPH Scavenging Activity

The antioxidant property of the 90% methanolic extract of leaves of *A. indica* was measured in vitro by 2, 2 diphenyl 1-picrylhydrazyl (DPPH) free radical scavenging assay according to Liyana-Pathiana and Shahidi [28]. DPPH (for 0.135mM) was dissolved in methanol. Plant extract dilutions ranging from concentration 40-400 µg/ml were prepared. One ml of 0.135mM DPPH and 1ml of plant extract of different concentrations were mixed in test tubes and incubated for 30 minutes at room temperature in dark. After incubation, the absorbance of the mixture was taken at 517nm using ELICO SL 210 UV-VIS spectrophotometer. Ascorbic acid was used as a standard. The DPPH scavenging activity was calculated as:

% DPPH scavenging activity
$= \{(Abs_{control} - Abs_{sample})/ Abs_{control}\} \times 100$
Where,
$Abs_{control}$ = Absorbance of methanol + DPPH
Abs_{sample} = Absorbance of sample (plant extract/ Ascorbic acid) +DPPH

Ferric Reducing Power Assay

The reducing power of methanolic extract of *A. indica* was evaluated by following the method of Oyaizu [29]. Plant extract having a concentration ranging from 200-400 µg/ml was prepared in distilled water. Plant extract (1 ml) of different concentrations was mixed with phosphate buffer (1.5 ml, pH 6.6. 0.2 M) and 1% Potassium ferricyanide (1.5 ml) and incubated in a water bath for 20 minutes at 50^0C. Followed by incubation, Trichloroacetic acid (10%, 1.5ml) was added and centrifuged at 4000 rpm using REMI R-8C laboratory centrifuge for 20 minutes. The supernatant (1.5 ml) was collected and mixed with distilled water (1.5 ml) and Ferric chloride (0.1%. 0.3 ml). The mixture was vortexed, and absorbance was taken using ELICO SL 210 UV-VIS spectrophotometer at 700nm.

Total Antioxidant Activity

The total antioxidant activity of *A. indica* was evaluated in vitro by following the procedure of Prieto [30]. Plant extract (0.1 ml) was mixed with 1.9 ml reagent (28mM NaH_2PO_4, 4mM Ammonium molybdate, and 0.6M H_2SO_4) and incubated in water bath at 95^0C for 90 minutes. After incubation, absorbance was taken at 695 nm using ELICO SL 210 UV-VIS spectrophotometer.

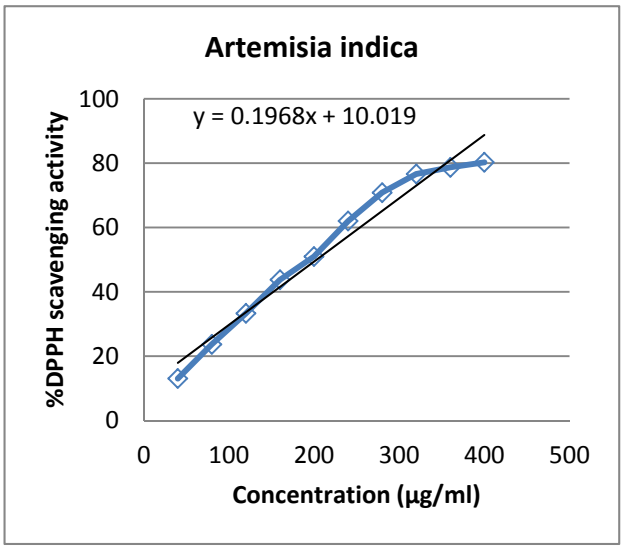

Figure 2: DPPH radical scavenging activity of *Artemisia indica*

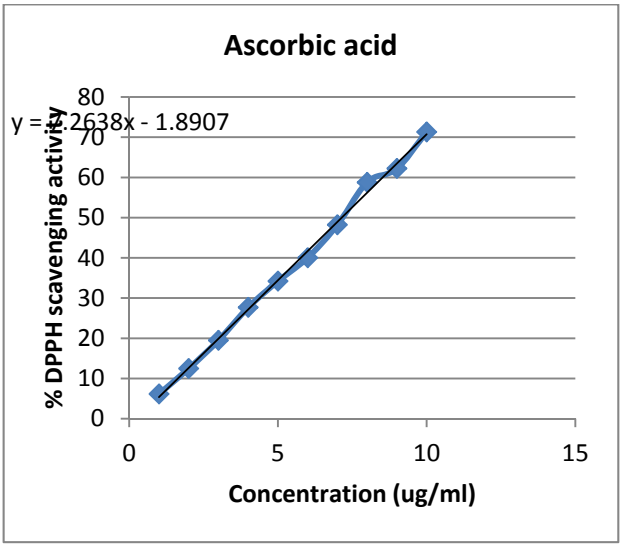

Figure 3: DPPH radical scavenging activity of ascorbic acid

EVALUATION OF ANTIBACTERIAL ACTIVITY

Test Microorganisms

Glycerol stocks of 5 bacterial strains namely *Escherichia coli, Staphylococcus aureus, Salmonella enterica, Pseudomonas putida* and *Streptococcus pyogenes* were obtained from Department of Microbiology, Lovely Professional University, Punjab, India. The bacterial strains were revived by inoculating 50 microliters of glycerol stock in 50-milliliter nutrient broth and incubated for 24 hours at 37^0C.

Antibacterial assay

Antibacterial activity of the plant extract was determined by agar well diffusion method [14]. Nutrient agar media was prepared, autoclaved and poured into Petri dishes to solidify. After solidification of the media, 100 microliters of bacterial culture was spread onto the agar media. Wells of diameter 6mm were made, and 80 microliters of the plant extract of concentrations 20 mg/ml, 50 mg/ml and 100 mg/ml were filled in the wells separately. Tetracycline (30 µg) susceptibility discs and DMSO were used as controls. Then, the plates were incubated for 24 hours at 37^0C. After 24 hours, zone of inhibitions around the wells were detected and their diameter was measured and recorded.

RESULT AND DISCUSSION

The treatment of various ailments using herbal medicines is a centuries-old tradition followed in India, China, and several other countries. These are the part of therapies including Ayurveda, Unani, and Siddha. One of the reasons behind their use is the presence of various phytochemicals having free radicals scavenging activity [12, 13]. Free radicals are produced in our body during normal metabolism for various purposes such as by macrophages for microbes killing. But, the excess production of these free radicals leads to the damage of the membrane and various macromolecules. It results with the onset of various ailments such as Parkinson, cancer, cardiovascular problems and Alzheimer [31-36]. Pollution, change in the lifestyle, smoking, alcohol, and reduction in the physical activity promote the production of more free radicals in the body. The antioxidant system in our body has ability to scavenge the free radicals, but excess free radicals still can harm our system. The use of plants and their products have been found to be effective in reduction of this oxidative stress [37].

Table 1: Antimicrobial activity of *Artemisia indica*

	Diameter in mm (20 mg/ml)	Diameter in mm (50 mg/ml)	Diameter in mm (100 mg/ml)
Gram-positive bacteria			
Streptococcus pyogenes	0	10	13
Staphylococcus aureus	12	14	18
Gram-negative bacteria			
Escherichia coli	11	12	15
Salmonella enterica	7	14	17
Pseudomonas putida	7	8	18

Not only the oxidative stress, the phytochemicals such as phenolic compounds, are responsible for showing the antimicrobial activity also [38]. In the today's world where the resistance towards antibiotic is increasing, the discoveries of novel compounds or preparation with anti-microbial activity is very much required. Thus, study on plants and their extracts for reducing the oxidative stress and to show the antimicrobial activity is an approach which is widely explored in the present scientific world. Keeping this in mind, we here tried to find out the effects of *Artemisia indica* alcoholic extract on oxidative stress and microbial growth.

Polyphenolic compounds

The presence of the polyphenolic compounds was measured by total phenolic compounds and flavonoids content. The total phenolic content of the methanolic extract of *A. indica* was found to be 76.30 µg equivalents of gallic acid per one gram of the plant extract. The presence of phenolic compounds such as phenolic acids, tannins, curcuminoids, quinines, etc. in a significant amount could be the important components for radical scavenging and antimicrobial activity [38]. Flavonoids are the critical group of polyphenolic phytochemicals that confer antioxidant and antimicrobial properties. The total flavonoid content for *A. indica* was found to be 14.33 µg equivalents of quercetin per 1000 µg of the plant extract. The presence of phenolics and flavonoids was an indication of having significant antioxidant and antimicrobial activity which was then measured experimentally.

Antioxidant status

For measuring the antioxidant status, the radical scavenging activity of the plant extract was measured on DPPH radicals. The DPPH free radicals scavenging activity of *A. indica* methanolic extract was found to be increasing with increase in the concentrations. Higher the amount of antioxidant in the plant extract, higher will be the DPPH scavenging ability. The IC_{50} value for the *A. indica* plant extract was found to be 204 µg/ml, and the IC_{50} value of ascorbic acid was 6.62µg/ml (Figure 2 and 3). Phenol and flavonoids whose presence was determined quantitatively in previous assays could be involved in showing DPPH radical scavenging activity. The IC_{50} value of plant extract as compared to ascorbic acid was significantly high. Ascorbic acid is a pure form of a phytochemical with potent radical scavenging activity. On the other hand, the plant extract is a mixture of compounds in which all the compounds do not show the radical scavenging activity. This difference could be the reason behind the significant difference between the IC_{50} value of plant extract and ascorbic acid.

In the determination of the reducing power of methanolic extract of *A. indica*, gallic acid was used as a standard compound. The reducing power of the 1000 µg methanolic extract was found to be equivalent to 13.40 µg of gallic acid. The plant extract was successfully able to reduce ferric chloride expectedly due to the presence of phenol, flavonoids and other phytochemicals. In the determination of the total antioxidant activity of the plant extract, ascorbic acid was used as a standard. The total antioxidant activity of 1000 µg of *A. indica* methanolic extract was found to be equivalent to 298.80 µg of ascorbic acid. The higher antioxidant activity of plant extract is relatable to the ability of plant extract to reduce ferric chloride and DPPH scavenging ability.

Antimicrobial activity

The antimicrobial activity of *A. indica* was determined against pathogenic and non- pathogenic bacteria. The plant extract showed antibacterial action against all the bacterial strains under study. The diameter of the zone of inhibition for *A. indica* (100mg/ml) was 15 mm, 18mm, 18 mm, 17 mm and 15 mm against *Escherichia coli, Staphylococcus aureus, Pseudomonas putida, Salmonella enterica and Streptococcus pyogenes*, respectively (Table 1). The treatment showed the effect in a dose-dependent manner with lowest for 25 mg/ml of plant extract. *A. indica* was found to have better antibacterial activity against *S. aureus*. The plant extract has shown the ability to inhibit the growth of both Gram-positive (*Streptococcus pyogenes* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli, Salmonella enterica*, and *Pseudomonas putida).*

CONCLUSION

Several studies have shown the potent antioxidant and antimicrobial activity present in various plants. Here, the extract of *Artemisia indica* revealed the presence of significant amount of flavonoid and phenol which contribute to its antioxidant activity. It was effectively able to inhibit the growth of both pathogenic and non-pathogenic bacteria in a dose-dependent manner. The study also corroborates the use of these plants in traditional medicine for the treatment of various diseases. Further investigation needs to be done to confirm the various pharmacological activities of compound present in *A. indica*.

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